

FUNCTIONAL DIVERSITY OF MICROBIOTA IN TYPICAL CHERNOZEM AT THE CULTIVATION OF SUGAR BEET

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A comparative analysis of number and diversity of microorganisms of main physiological groups in typical chernozem in the main phase of the ontogeny of sugar beet under different agrarian systems and methods of soil tillage is conducted. The structure of different taxonomic groups and qualitative composition of the ecological indices is shown.

Soil is the main reservoir and the natural habitats of microorganisms, which are characterized by a wide specific and functional diversity [1]. The number, structural organization and functional properties of the microbiota are the basic genofund at the formation of soil, its structure and fertility [2]. According to the latest scientific data of scientists' microbiologists, the number of microorganisms in the soil can reach several billion in 1 g of soil, and total biomass - up to 10 t/hectare.

Vital activity of microbiota in soil, its qualitative and quantitative structure, the ratio of physiological groups of microorganisms is defined of soil conditions (soil type, availability of nutrients, moisture, aeration, pH, temperature, etc.), biotic and abiotic factors [3-5]. Anthropogenic factors have a considerably affect at the microbial communities (type and norms of fertilizers, methods of soil tillage, permanent cultivation of crops and crop rotation application, the use of plant growth regulators, application of pesticides) [6].

It is known that the quantitative and qualitative composition of soil microbiota is a sensitive indicator of agroecosystem condition and it reflects the degree of anthropogenic impact. So it is used as a diagnostic indicator at an estimation of soil ecological condition, which makes it possible to detect any changes of the ecosystem at early stage [4, 6-8]. Thus, it is necessary to take into account major role of microorganisms that have high sensitivity to environment changes and adaptive sustainability and multifunctionality [7, 8]. Therefore, the researches of quantitative and qualitative structure of microbial groups of anthropogenic modified agroecosystems are necessary task for understanding the mechanisms of interaction between the components of soil microbiota, the impact of soil environmental factors on biological processes, soil properties, agrotechnical actions, agrarian systems and soil management processes.

The object of research is to study the features of formation of the structure and biodiversity of microbial complex in typical chernozem at the sugar beet cultivation under various agrarian systems and methods of soil tillage in the main phase of culture ontogeny.

Research methods. The researches of microbiota of typical chernozem are conducted at the stationary field experiment of the department of agriculture and herbology of NULES of Ukraine «Agronomic Research Station» in the forest-steppe zone of beet-grain crop rotation. The sampling of soil was carried out from the top of the arable root layer (0-20 cm) in the phase of coming-up of sugar beet (*Beta vulgaris*) and in the phase of leaves closure in row-spacing.

The scheme of the experiment is included to study of 3 agrarian systems (AS) and 2 soil tillage (ST), as a whole 6 variants of the experiment:

1) industrial AS - (control) - (application of 300 kg NPK of fertilizer, 12 tons of manure per hectare of crop rotation, intensive application of chemical measures of plants protection) + surface ST (cultivation of disk tools to a depth of 8-10 cm under all crops rotation); 2) industrial AS + differentiated ST (to hold 6 times plowing on different depth, 2 times the surface tillage under winter wheat after peas and corn silage and 1 time - the tillage under barley, of crop rotation); 3) ecological AS (application of 150 kg NPK of fertilizer, 24 tons of organic fertilizer (12 tons of manure, 6 tons of non-commercial harvest, 6 tons of green manure crop mass) per hectare of crop rotation, the use of chemical and biological plant protection agents on the criterion of ecological and economic threshold of presence of harmful organisms) + surface ST; 4) ecological AS + differentiated ST; 5) biological AS (application only organic fertilizers (24 tons), the use of biological measures of crop protection) + surface ST; 6) biological AS + differentiated ST [9].

The number of microorganisms of the basic physiological and taxonomic groups was determined by inoculation of soil suspensions on solid culture mediums [3]. The qualitative structure of the microbial complex were studied on the basis of cultural representation of morphological types [10]. The ecological indexes of Shannon, Simpson and Berger-Parker were determined to the ecological condition of microorganisms in the soil [11]. Statistical analysis of research results were conducted in Ms Excel.

Results. Researches of microbiota of typical chernozem are established significant changes in the formation of the number of basic physiological groups of microorganisms that connected with the changes of soil nutrient regime and its properties, which are caused by application of agrarian systems and methods of soil tillage (table 1).

1. The number of physiological groups of microorganisms in typical chernozem in sugar beet ontogeny

Physiological groups of microorganisms		Experiment variants					
		1	2	3	4	5	6
		Sur. ST	Diff. ST	Sur. ST	Diff. ST	Sur. ST	Diff. ST
		Industrial AS		Ecological AS		Biological AS	
		million CFU/1 g. a.d.s.					
Phase of coming-up	Ammonifying	4,31±0,32	10,25±0,55	9,84±0,39	6,34±0,28	7,99±0,55	10,49±0,73
	Nitrifying	6,34±0,28	9,68±0,85	13,86±0,85	9,03±0,27	11,62±1,09	11,59±1,37
	Oligotrophic	8,21±0,84	9,10±0,74	16,56±0,43	6,43±0,42	7,58±0,84	12,44±1,00
	Pedotrophic	7,73±0,42	9,43±0,70	8,28±0,58	4,07±0,57	7,99±0,55	8,95±0,57
	Oligonitroifying	5,73±0,59	7,95±0,32	12,30±0,84	4,80±0,57	6,78±0,54	11,63±1,42
	Phosphate immobilizing	8,78±0,83	10,09±0,84	6,40±0,56	7,65±0,42	10,89±0,55	11,35±0,42
	Streptomycetes	0,81±0,16	0,82±0,16	1,64±0,43	1,30±0,16	0,89±0,16	0,73±0,00
	Sporeforming	2,28±0,42	0,98±0,28	1,89±0,32	1,55±0,16	1,86±0,42	2,52±0,42
	Cellulolytic*	43,5±3,07	55,76±3,76	65,60±2,56	57,34±3,45	76,23±4,19	81,33±5,77
	Micromycetes*	17,08±2,76	24,19±1,61	38,13±2,78	28,06±2,76	27,83±1,40	31,31±2,11
Phase of leaves closure in row-spacing	Ammonifying	18,76±1,55	19,33±1,83	13,69±1,49	14,97±1,33	22,37±1,58	15,23±0,67
	Nitrifying	16,47±2,24	15,83±1,22	10,94±1,67	21,17±1,47	28,91±1,61	21,51±0,41
	Oligotrophic	5,95±1,10	7,08±1,03	7,27±1,08	6,05±0,46	23,07±2,12	4,71±0,53
	Pedotrophic	21,2±1,72	18,95±0,65	14,91±2,08	18,61±1,86	28,79±2,10	14,68±0,81
	Oligonitroifying	8,62±0,83	13,32±1,72	8,57±1,17	17,30±0,55	26,16±2,00	7,77±0,46
	Phosphate immobilizing	12,66±1,87	16,21±1,95	14,07±0,83	18,61±1,50	32,28±1,51	15,93±0,56
	Streptomycetes	1,60±0,26	0,68±0,26	0,99±0,15	0,39±0,15	0,00	1,88±0,27
	Sporeforming	5,80±0,54	5,33±0,15	4,59±0,52	3,26±0,26	5,81±0,26	3,22±0,41
	Cellulolytic*	40,79±5,23	51,74±3,95	61,18±2,70	54,68±3,72	73,14±4,55	78,50±4,07
	Micromycetes*	16,01±2,59	22,45±2,69	35,56±3,43	26,76±3,48	26,70±1,31	30,22±3,35

* - thousand CFU/1 g. a.d.s.

In the phase of coming-up of culture the number of bacteria of a large part of the main physiological groups is increased at the application of biological and ecological AS in comparison with the industrial. Thus, the number of oligotrophic microbiota is increased by 15,6 and 32,8 % (at the application of biological and ecological AS, as compared to industrial), ammonifying – by 26,9 and 11,1%, nitrifying - by 44,9 and 42,9 %, oligonitroifying - by 34,5 and 24,9 %, sporeforming - by 34,2 and 5,2 %, cellulolytic - by 58,7 and 23,8 %, micromicetes - by 43,3 and 60,4 % respectively. The number of phosphate immobilizing microorganisms is grew to 17,9 % at the biological, and to 80,1 % at the ecological AS as compared with industrial AS. This indicates that favorable conditions for the optimal functioning of soil microbial coenosis creates at the use of organic matter (manure, crop remnants, green manure) due to the application of biological and ecological AS. The number of pedotrophic microbiota, however, was raised in the conditions of application of industrial AS, due to their trophic specificity and lack of interspecific competition.

The method of soil tillage also had a significant impact on the quantitative composition of the soil microbial complex. Thus, the number of a large part of the physiological groups of microorganisms was increased at the surface ST by 1,8-19,2 % as compared to differentiated ST (except ammonifying, phosphate immobilizing and cellulolytic microorganisms, whose number was decreased by 4,7 – 18,3 %) at the beginning of sugar beet vegetation, that testifies to increase of intensity of mineralization processes of nitrogen-containing compounds. Soil tillage had no impact on the number of micromicetes.

In the phase of leaves closure in row-spacing the number of ammonifying, nitrifying (except variant ecological AS + surface ST), oligonitroifying (except ecological AS + surface ST and biological AS + differentiated ST), phosphate immobilizing, pedotrophic, sporeforming microorganisms was increased in 1,28-5,41 times. The number of micromicetes, cellulolytic microorganisms, however, was decreased by 3,5-7,2 %, oligotrophic (except biological AS + surface ST) and streptomycetes (except industrial AS + surface ST) - by 22,2-70,2 %. This results from the fact that the soil microflora (including the influence of plant exudates), processes of transformation of organic substance becomes more active in active vegetation of plants.

In general, the total number of all microorganisms in soil layer 0-20 cm during culture vegetation was the highest at the biological AS. Thus, the total number of microbiota at this AS in phase of coming-up of culture was on 20,4 % higher than at the ecological and on 40,2% than at the industrial AS, in the phase of leaves closure in row-spacing– on 43,5 and 51,6 % respectively. Application of differentiated ST is promoted to increase the total number of all microorganisms of the soil in the beginning of sugar beet vegetation by 2,2%, in the period of active vegetation - by 18,0 %. That is, the application of this agrarian measures are increased the content of readily available organic compounds that can be a source of energy for soil microbiota.

Analysis of the qualitative structure of the soil bacterial and fungal microflora at the application of various agrarian systems and methods of soil tillage has shown

significant differences of the number of identified morphotypes and the structure of distribution of dominant forms of microorganisms (fig. 1, 2).

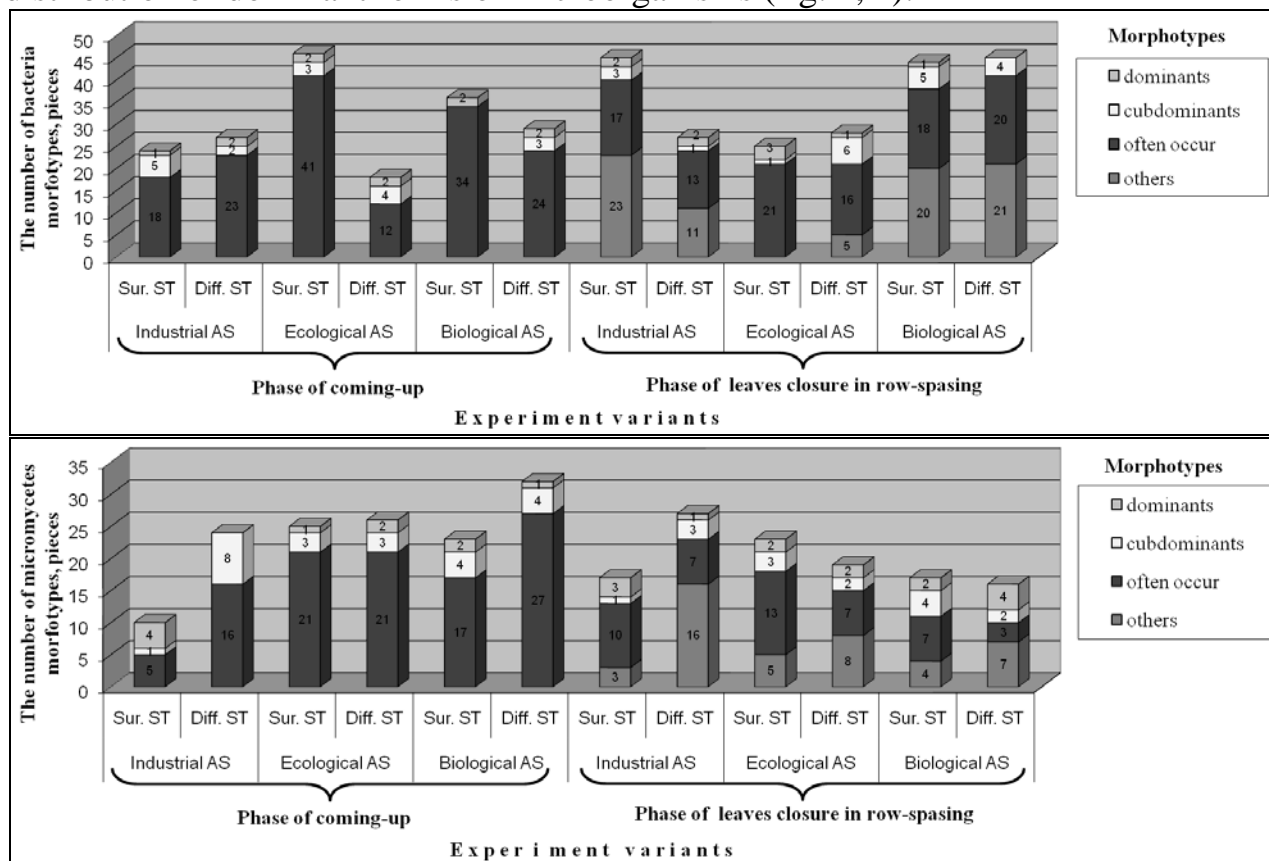


Fig. 1 The qualitative structure of microbial complex in typical chernozem

Thus, more diversity of morphotypes in the phase of coming-up was at the application of biological and ecological AS, the number of bacteria in which was higher by 27,5 and 24,5 %, as compared with the industrial AS, micromycetes – respectively by 61,8 and 50,0 %. There was a redistribution of microbial coenosis structure in the phase of leaves closure in row-spasing: the total number of morphotypes of bacteria was the largest at the biological AS (it is increased by 36,9 % in comparison with the beginning of vegetation), and the diversity of micromycetes was decreased (by 66,7%). Thus, the total number of bacterial morphotypes at the beginning and in the middle of culture vegetation was higher by 43,2 and 14,0 % at the application of surface ST in comparison with the differentiated ST, while the number of micromycetes was decreased by 29,3 and 8,7 %. The largest share of the microbiota were morphotypes that «often occur» (50,0-94,4 % were in the phase of coming-up and 6,7-84,0 % were in the phase of leaves closure in row-spasing), the share of «other» morphotypes in the phase of active vegetation of culture were 17,6-56,5 % (except experiment variant ecological AS + surface ST and in the phase of coming-up at which they were outside of detection).

The analysis of dominant morphotypes has been carried out to the estimation of a microbic complex. This list of the dominants is one of representative indicators of taxonomic structure of microbial systems, closely connected with the formed type of agroecosystems [6]. There were found 4 dominant forms of bacteria, 9 dominants of micromycetes in the phase of plants coming-up. In the phase of leaves closure in row-

spacing it was a redistribution of structure of dominant by the increasing of the bacteria number to 9 units and reducing of micromycetes to 6 units. This is indicated at the impoverishment of bacterial diversity by increasing the level of dominance and enrichment of micromycetes during the active crops vegetation. The largest number of dominant micromycetes (4 pcs.) was found in the experiment variant industrial AS + surface ST at the beginning of vegetation. It testifies to formation of a homogeneous microbial complex of typical chernozem with a high degree of dominance of fungal microflora. Distribution of bacterial morphotypes was uniform (2 pcs.) except variant of industrial AS + surface ST (1 pcs.). The most micromycetes were found at the surface ST during the active vegetation of culture. It should be noted that a dominant position in all experiment variants in phase of coming-up was the morphotype number of 1 of bacterial microflora (except ecological AS + surface ST), and in the phase of leaves closure in row-spacing was morphotype number of 2 of fungal microflora (fig. 2).

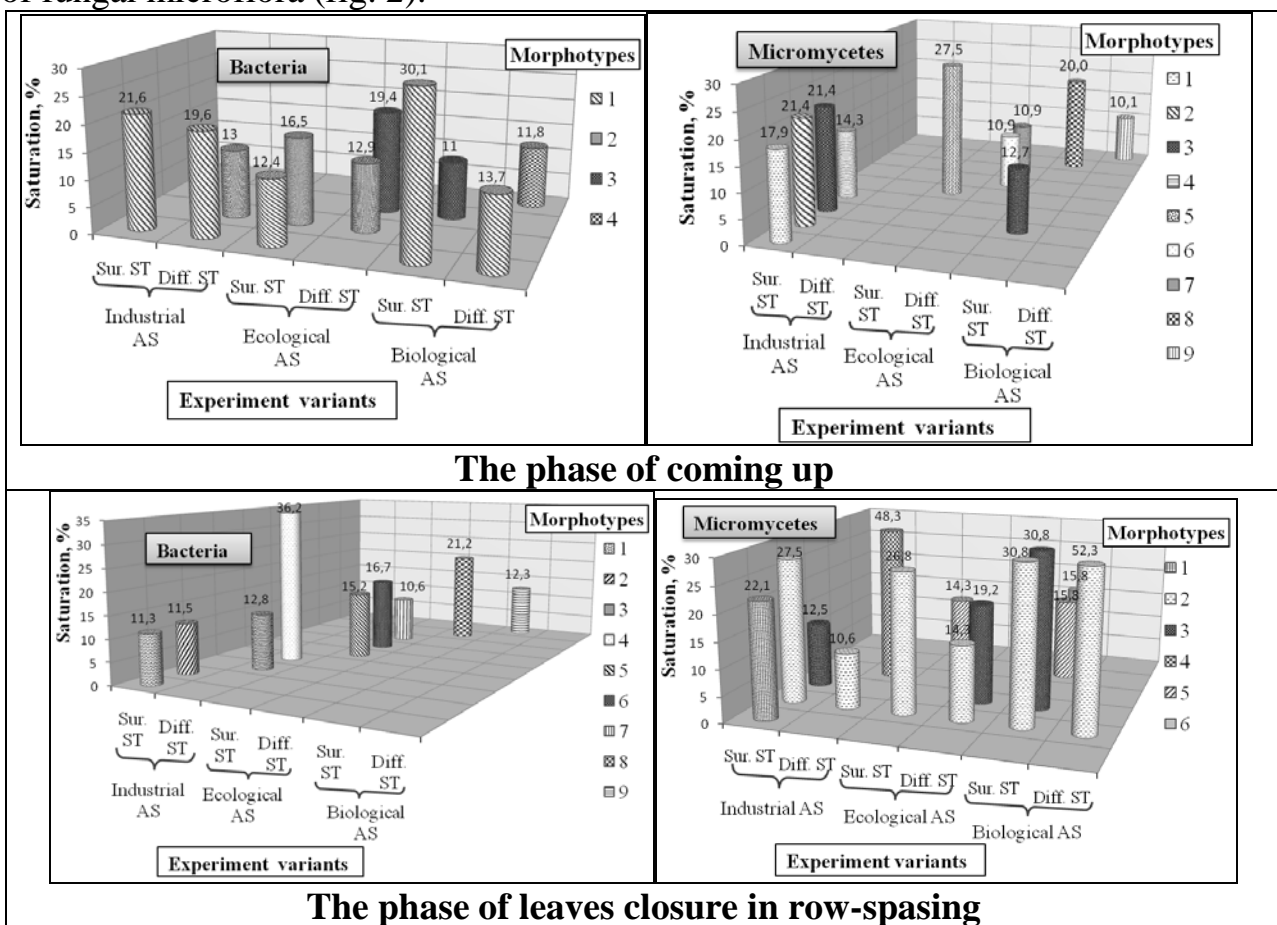


Fig. 2. Distribution of dominant morphotypes of fungal and bacterial microflora of chernozem typical at various agrarian measures

The magnitude of the Shannon index is established that favorable conditions for the functioning of microbiota were created in the soil during the vegetation at the biological AS, because there were the highest indicators of microflora biodiversity at this agrarian system (by 1,1-20,5 % more than at the ecological and industrial AS) (Fig. 3). Application of surface ST also contributes to increase the biodiversity of microflora by 5,2-6,0 %, compared with the differentiated ST, due to the localization

of organic residues and fertilizers in the soil layer. However, the micromycetes diversity was decreased by 21,2 % in the phase of coming-up of culture at the application of this ST due to the peculiarities of their metabolism.

The increase of Simpson index of micromycetes in phase of coming-up (0,12) at the application of industrial AS with the surface ST and bacteria in the phase of leaves closure in row-spacing (0,16) at the industrial AS with the differentiated ST are indicated to decrease of biodiversity in the soil. It is necessary to notice that there are existed a clear inverse relationship between the indexes of Shannon and Simpson that testifies to formed systems of microbial communities.

The greatest domination index of Berger-Parker was discovered at the industrial AS, while at the beginning of the biodiversity it was lower at the surface ST and during the active vegetation - at the differentiated ST. Between Berger-Parker's index and Simpson's ($r = + 0,55-0,93$) and Shennon's indexes ($r = - 0,59-0,97$) were existed the close correlation, which is confirmed previously obtained results. That is, the greatest diversity of bacterial and fungal microflora with the lowest degree of dominance of one species was discovered at the biological AS.

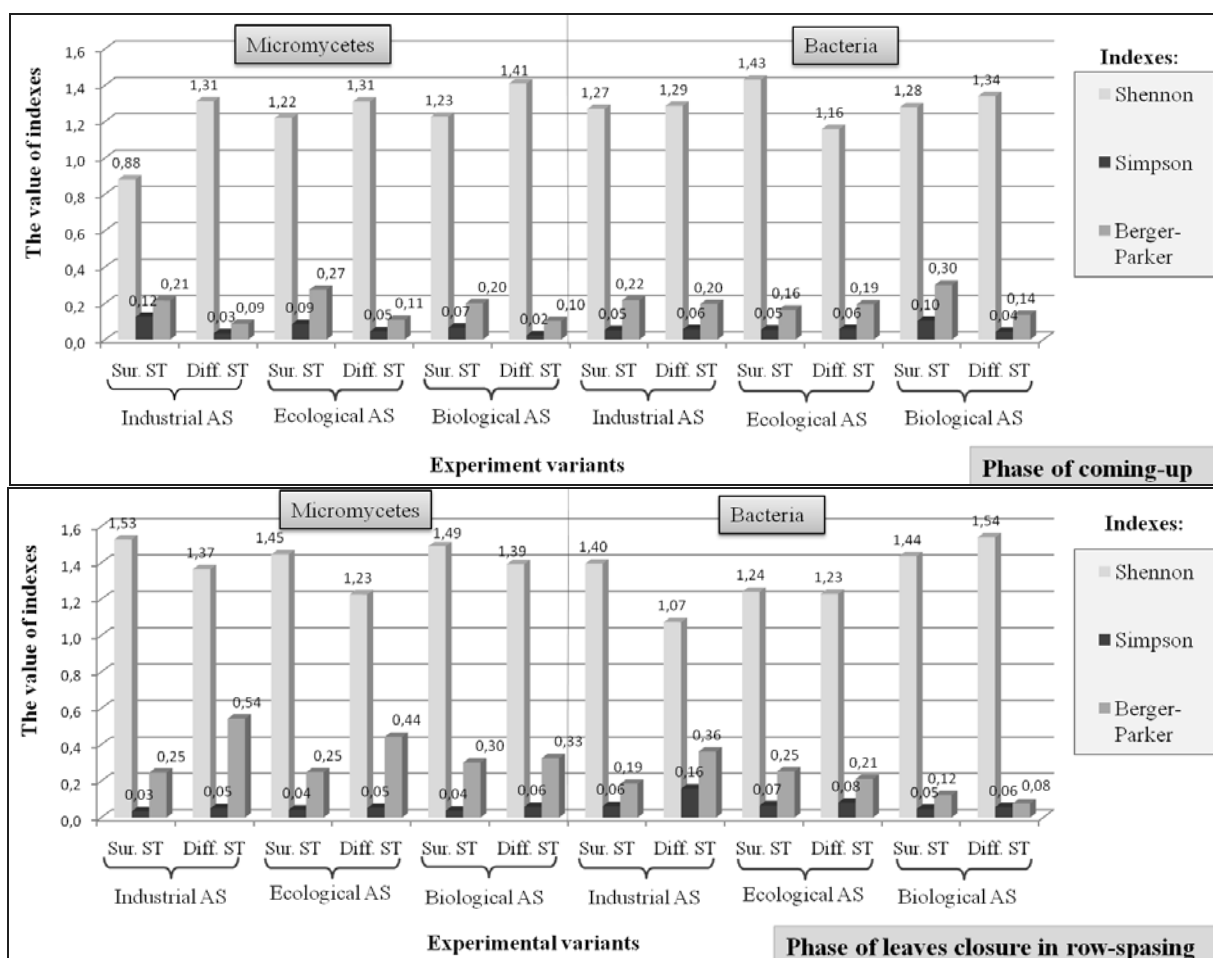


Fig. 3. Ecological characteristics of chernozem typical at the sugar beet cultivation

Conclusions. Thus, the number, the quality, the structure and the ratio of various physiological groups of soil microorganisms depends on the number, type of fertilizers (organic, mineral) and soil tillage. Applying of considerable quantity of

mineral fertilizers leads to qualitative and quantitative changes in the microbial complex of typical chernozem, that is accompanied by a simplification of trophic connections and biodiversity decrease. Application of biological AS promotes compensation of basic nutrients of organic nature thanks to receipt of organic fertilizers. It is cause the activation of microbiological activity of the soil by increasing abundance and biodiversity of the microbiota, expansion of the trophic connections of microbial cenosis and, ultimately, the formation of homeostatic microbial biomes of soil ecosystems.

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**Функциональное разнообразие микробиоты чернозема типичного при
выращивании сахарной свеклы**

Ключевые слова: микроорганизмы, сахарная свекла, чернозем типичный, системы земледелия, обработка почвы

Микробиота – индикатор состояния экосистемы, изменения количественного и качественного состава которого указывает на степень воздействия биотических, абиотических и антропогенных факторов на состояние почвенной экосистемы в целом. Установлено, что при внесении значительного количества минеральных удобрений уменьшается численность, биоразнообразие, изменяется качественный состав и соотношение различных физиологических групп микроорганизмов. За счет внесения органических удобрений происходит оптимизация микробиологической деятельности почвы и формируются устойчивые гомеостатические системы микробных ценозов.

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**Функціональне різноманіття мікробіоти чорнозему типового при
вирощуванні буряка цукрового**

Ключові слова: мікроорганізми, буряк цукровий, чорнозем типовий, системи землеробства, обробіток ґрунту

Мікробіота – індикатор стану екосистеми, зміна кількісного і якісного складу якого вказує на ступінь впливу біотичних, абіотичних і антропогенних факторів на стан ґрунтової екосистеми в цілому. Встановлено, що при внесенні значної кількості мінеральних добрив зменшується чисельність, біорізноманіття, змінюється якісний склад і співвідношення різних фізіологічних груп мікроорганізмів. За рахунок внесення органічних добрив відбувається оптимізація мікробіологічної діяльності ґрунту і формуються стійкі гомеостатичні системи мікробних ценозів.